REMARKS

A check for the fee for a two month extension of time to render the response timely filed is enclosed. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-3, 5-10, 14, 15, 17-33 and 42-44 are pending. Claims 1, 9, 10, 17 22-24, 27 and 30 are amended and claims 12 and 16 are cancelled without prejudice or disclaimer. Claim 1 is amended by incorporation of claim 16, which is cancelled herein without prejudice. Claim 1 also is amended to render it clear that a property, such as stability, or activity, such as enzymatic activity, of a protein can be evolved. Claim 1 is amended and each of independent claims 22-24, 27 and 30 are amended to render it clear that amino acids are replaced one-by-one along the full length of the protein to be evolved or along the full length of a domain of the protein. In addition, these claims are amended to recite "whereby the identity of each set of nucleic acid molecules in host cells at each locus in the array is known" to render it clear that as intended in the claim and by virtue of the method an **array** is produced in which the **identity of the members is known *a priori*.**

The **Examiner** comments in the Office Action that the specification only states that an addressable array is one in which the a locus is identifiable. To clarify the step in the method, the claim is amended to render it clear that the steps of the method result in an array in which the identity of the encoded protein at each locus is known. In this instance, the identity is known by virtue of method in which each amino acid is systematically replaced one-by-one and each modified protein, which has a single amino acid change is separately expressed in a host cell. Thus, in the claimed methods, sets of nucleic acids encoding proteins that differ in one amino acid from the target protein are prepared, the sets are individually expressed and screened to identify modified proteins that have a predetermined activity. As noted previously, the methods involve modifying nucleic acid molecules one codon at a time to replace a single amino acid at a time <u>along the full length of the protein or along the full length of a domain thereof so that all positions along the full-length or a domain of the protein are individually modified for screening</u>, introducing modified nucleic acid molecules one-by-one into host cells to produce addressable arrays in which the identity of each encoded protein is *a priori* known, and individually screening each protein that is produced. Each modified protein is produced, expressed and screened separately; a plurality

of proteins are produced so that all hit positions along the full-length or a domain of the proteins are identified. Each modified protein is produced separately and is separately screened. No new matter is added.

An Information Disclosure Statement is being filed under separate cover on the same day herewith.

THE REJECTIONS OF CLAIMS 1-21 AND 42-44 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-10, 12, 14-33 and 42-22 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for reasons addressed in turn below for failing to particularly point out and distinctly claim the subject matter that applicant regards as the invention. This rejection is respectfully traversed.

1. Claims 1, 22-24, 7 and 30 are rejected as failing to provide proper antecedent for "the cells of each locus of the addressable array contain the same modified nucleic acid molecule" since prior recitations of "nucleic acid molecule" were plural. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendment to change the singular to plural. It is noted that claim 30 does not recite this language.

2. Claims 1, 22-24, 7 and 30 are rejected in the recitation of "each such protein is designated a hit" because the antecedent for "such protein" allegedly is unclear. For clarity the claims are amended to recited "each identified protein is designated a hit," which refers to the previous recitation of the protein, which states "to identify one or more proteins."

THE REJECTIONS OF CLAIMS 7, 24-29, 32, 33 UNDER 35 U.S.C. §103(a)

Several grounds of rejection are set forth under 35 U.S.C. §103(a) The particular grounds of rejection are discussed in turn below. As discussed below, neither Blazquez *et al.*, nor Giver *et al.* nor any reference of record, nor any combination of references, teaches or suggests steps (a) -(d) of the methods as outlined above and as recited in each of the independent claims. None of the references teaches or suggests changing one amino acid at a time along the full length of the protein or domain thereof so that all positions along the full-length or a domain of the protein are individually modified for screening and all hits along the full-length or domain are identified. None suggest separately introducing each nucleic acid molecules cells of one locus of an array to produce an addressable array in which the identity of the encoded protein is known *a priori*.

In addition, as exemplified in the application, the methods of the instant application are very powerful, permitting preparation of modified polypeptides that have a predetermined property or activity. In the working example in the application, the overlapping Rep protein-encoding gene(s) of AAV are changed one codon at a time along the full-length of the open reading frames encoding the Rep proteins to identify loci (hits) whose modification alters AAV titer. Each hit is then changed to every other amino acid to identify modified proteins that result in higher titer. The methods identifies at least 6 such proteins in each serotype. Heretofore, no Rep mutants had ever been identified that result in increased titer. Hence the method permits evolution of protein (*i.e.*, Rep proteins) to exhibit a predetermined property (*i.e.*, higher titer).

As discussed below, the Blazquez *et al.* fails to teach several steps or elements of the methods as claimed. For example, Blazquez *et al.* does **not teach** a method of directed evolution, does **not** teach a method that includes steps of modifying each amino acid along the full length of a protein or domain thereof to identify hit loci, nor does Blazquez *et al.* teach introduction of modified nucleic acid molecules into host cells such that the identity of the encoded protein at each locus of the array is *a priori* known. Blazquez *et al.* assesses the effects on a protein of naturally-occurring mutations. Mutations are not made one-by-one along the full-length or domain of a protein.

Giver *et al.* does not cure these deficiencies. As discussed in the previous response, the method of Giver *et al.* necessarily produces **a mixture** of nucleic acid molecules and also of expressed proteins. The instant claims require production and screening of single proteins. Further Giver *et al.* introduces **one to two** mutations per molecule, **not one** as required by the instant claims **and** Giver *et al.*, does not introduce mutations along the full length of a protein or domain thereof. In the method of Giver *et al.* mixtures of nucleic acid molecules are introduced into cells, the cells are grown and colonies picked and screened. The polypeptides that are selected as having a property of interest must be sequenced to identify hit positions. Thus, Giver *et al.* does not teach a method in which cells are arrayed such that the identity of the encoded protein at each locus is known. In the method of Giver *et al.* the identify of the encoded protein in the picked colony is not known. The method of Giver *et al.* does not identify the protein and a hit position in a single step as in the instantly claimed methods.

None of the secondary references nor any reference of records provides any suggestion to change the operation of the method of Blazquez *et al.* and/or the method of

Blazquez *et al.* in combination with the method of Giver *et al.* None provide any suggestion to modify the method of Blazquez *et al.*, which assesses the effects of the amino acid differences in naturally-occurring TEM-β-lactamases.

RELEVANT LAW

In order to set forth a prima facie case of obviousness under 35 U.S.C. § 103: the combination of the cited references must actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art." In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (ACS Hosp. Systems, Inc. v Montefiore Hosp. 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

Importantly, all claim limitations must be taught or suggested by the prior art to establish that claims are *prima facie* obvious. See, e.g., MPEP 2143.03 and In re Lowry, 32 F.3d 1579, 32 U.S.P.Q.2d 1031 (Fed. Cir. 1994), citing In re Gulack, 703 F.2d 1381, 217 U.S.P.Q. 401 (Fed. Cir. 1983), citing In re Royka, 490 F.2d 981, 180 U.S.P.Q.2d 580 (CCPA 1974).

The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, In re Papesh, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). In addition, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. In re Ratti, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

The question of obviousness is resolved on the basis of underlying factual determinations including (1) the scope and content of the prior art, (2) any differences

between the claimed subject matter and the prior art, (3) the level of skill in the art, and (4) where in evidence, so-called secondary considerations. Graham v. John Deere Co., 383 U.S. 1, 17-18 (1966). See also " KSR Int'l Co. v. Teleflex Inc., 127 S. Ct. 1727, 1734 (2007). ("While the sequence of these questions might be reordered in any particular case, the [Graham] factors continue to define the inquiry that controls.")

In KSR, the Supreme Court emphasized that "the principles laid down in Graham reaffirmed the 'functional approach' of Hotchkiss, 11 How. 248 [(1850)]." KSR, 127 S. Ct. at 1739 (citing Graham v. John Deere Co., 383 U.S. 1, 12 (1966) (emphasis added)), and reaffirmed principles based on its precedent that "[t]he combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." Id. The Court explained: When a work is available in one field of endeavor, design incentives and other market forces can prompt variations of it, either in the same field or a different one. If a person of ordinary skill can implement a predictable variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill. Id. at 1740. The operative question in this "functional approach" is thus "whether the improvement is more than the predictable use of prior art elements according to their established functions." Id.

The Supreme Court stated that there are "[t]hree cases decided after Graham [that] illustrate this doctrine." Id. at 1739. "In United States v. Adams, … [t]he Court recognized that when a patent claims a structure already known in the prior art that is altered by the mere substitution of one element for another known in the field, the combination must do more than yield a predictable result." Id. at 1740. "Sakraida and Anderson's-Black Rock are illustrative – a court must ask whether the improvement is more that the predictable use of prior art elements according to their established function." Id. at 1740.To be nonobvious, an improvement must be "more than the predictable use of prior art elements according to their established functions." Id. at 1740.

In the instant case, the combination of teachings of the references does not teach or suggest all elements of the methods as claimed, hence the issue of whether the claims are more than a predictable use of prior art elements is not even reached.

Rejection of claims

Claims 1-3, 5, 6, 8-10, 12, 14-23, 32 , 33 and 42 are rejected under 35 U.S.C. §103(a) as being unpatentable over Blazquez *et al.* in view of Giver *et al.* Blazquez *et al.* allegedly teaches every element of the claimed method except that the host cells are organized into an array, but Giver *et al.* allegedly cures this defect. This rejection respectfully is traversed.

THE REJECTED CLAIMS

Independent claim 1 is directed to a process for the identification of a protein that differs in a predetermined property or activity from a target protein, comprising:

(a) producing a plurality of separate sets of nucleic acid molecules that encode modified forms of a target protein, wherein:

the nucleic acid molecules in each set are produced by changing one codon in the target protein to a pre-selected codon, whereby the nucleic acid molecules in each set encode proteins that differ from the encoded proteins in another set by one amino acid;

the encoded amino acid residues are each replaced along the full-length of the encoded protein or along the full-length of a pre-selected domain of the encoded protein so that all positions along the full-length or a domain of the protein are individually modified for screening; and

all nucleic acid molecules in a set encode the same modified protein;

(b) individually introducing each set of nucleic acid molecules into host cells to produce host cells that are organized in an addressable array, whereby the identity of each set of nucleic acid molecules in host cells at each locus in the array is known, wherein the cells of each locus of the addressable array contain the same modified nucleic acid molecule molecules;

(c) expressing the encoded proteins, whereby a plurality of separate sets of proteins encoded by the nucleic acid molecules are produced, wherein:

all of the encoded proteins in each set have the same modification; and

the proteins in each set differ from the proteins in another set by one amino acid and from the target protein by one amino acid; and

(d) individually screening each set of encoded proteins, whereby to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified, wherein:

each such identified protein is designated a hit;

each hit contains a mutation designated a hit position; and

the predetermined property or activity is selected from among a chemical, a physical and a biological property or activity of the target protein.

Claim 22 is directed to the process for the identification of a protein that differs in a predetermined property from a target protein by individually expressing a plurality proteins that differ by one amino acid and individually screening them. Claim 22 recites that the

predetermined property is selected from among a chemical, a physical and a biological property of the target protein, wherein the change in a predetermined property comprises a change in an activity of the target protein that is at least about 10%, 20%, 30%, 40% or 50% compared to the unmodified target protein. Claim 23 recites that the change in the predetermined property comprises a change in an activity of the target protein that is at least about 75%, 100%, 200%, 500% or 1000% compared to the unmodified target protein. Claim 24 and dependent claims recite that the "predetermined property is selected from among a chemical, a physical and a biological property of the target protein". Claim 27 and dependent claims recite that the "nucleic acid molecules comprise viral vectors; and the cells are eukaryotic cells that are transduced with the vectors," and claim 30 and dependents recite that "performance of the screened proteins is evaluated by a Hill analysis or by fitting the output signal to a curve representative of the interaction of the target protein and a test compound."

Hence all of the claims are directed to method of directed evolution in which a target protein is modified one amino acid at a time along its full length or along the full length of a domain, and each modified protein is separately expressed and screened separately from the other modified proteins. One amino acid change is introduced per protein, not one or more changes, but one change. Mixtures of different proteins are not produced, nor are mixtures screened. In addition, because one change is made at a time and the nucleic acid molecules encoding each protein are introduced host cells at different loci in an array the identity of the nucleic acid at each locus in the array, and, thus the protein, is known.

As described in the application this method does not rely on any methods in which there is differential modification or expression of a particular modified protein. For example, at page 24, lines 6-24, the specification states:

> The whole process of the 'identification of the active site(s) on the full length protein sequence requires the following sub-steps:
>
> a. Generation of a mutant library (on the gene to be evolved) in which each individual mutant contains a single mutation located at a different amino acid position and that includes a systematic replacement of the native amino acid by Ala or any other amino acid (always the same throughout the entire protein sequence);
>
> b. phenotypic characterization of the individual mutants, one-by-one and assessment of mutant protein activity;
>
> c. identification of those mutants that display an alteration, typically a decrease, in the selected protein activity, thus, indicating that amino acids directly involved in the active site(s) have been hit. The aa

> positions whose aa-scan mutations display an alteration, typically a loss or decrease, in activity are named HITS.
>
> The identification of the active site(s) (HITS) is thus, by this method, made in a completely unbiased manner. There are no assumptions about the specific structure of the protein in question nor any knowledge or assumptions about the active site(s). The results of the amino acid scan identify such sites.

Hence, there is no bias introduced into the process. Amino acids are rationally replaced and each variant is tested separately. There is no reliance upon differential expression in culture in which conditions could favor one variant over another. Further, as exemplified, the method is quite powerful, permitting evolution, for example, of AAV Rep proteins to produce modified AAV that has that has increased titer. While modifications in the viral Rep genes was known, there had been no mutations identified that result increased titer. The instantly claimed unbiased systematic rational method permitted mutations that result in increased titer to be identified.

The Examiner has mischaracterized the claim

As noted above, the Examiner states that:

> [t]he instant claims are drawn to a method of identifying proteins with different properties by producing a set of nucleic acid molecules that encode modified proteins that differ from a target protein by one amino acid; introducing the nucleic acids into host cells on a array; expressing the proteins; screening the proteins for a chemical, physical, or biological property that differs from the target protein; and designating proteins with a different property from the target protein as a hit.

It respectfully is submitted that this characterization of the claims is incorrect. The instantly claimed methods produce separate sets of nucleic acid molecules. Each set encodes the same protein, and the protein encoded by each set differs from each other set by one amino acid. Hence, the method does not include a step in which mixtures of nucleic acid molecules that encode different proteins are prepared. Further, as claimed, the sets are individually introduced into host cells and each set is expressed to produce one protein at each locus of an array. Each protein is separately screened.

Differences between the teachings of the cited references and the rejected claims

Blazquez *et al.*

Blazquez *et al.* teaches assessment of the contribution of amino acid substitutions at only **seven** positions altered **in naturally-occurring TEM β-lactamases** to determine the contribution of each amino acid to the antibiotic-resistant phenotype. Blazquez *et al.* teaches that production of TEM-type β-lactamases is the most prevalent mode of resistance to

β-lactam antibiotics and that resistant variants result from alteration of one or combinations of specific amino acid substitutions in seven loci (Gln 39, Glu104, Arg 164, Ala237, Gly238, Glu240 and Thr265). To study the effects of the mutations, Blazquez *et al.* constructs seven different muteins, each with a single change and studies the effects of the change on phenotype. Blazquez constructs seven modified polypeptides with mutations at seven different, non-contiguous loci. Blazquez *et al.* simply produces seven different polypeptides and tests their activity. In Blazquez *et al.* the **hits (sites that affect an activity or property of the protein) are known**.

Hence Blazquez *et al.* **does not** teach or suggest any method in which a target protein is modified to have a predetermined activity or property. Blazquez *et al.* tests the effects of known mutations to assess their effects on phenotype, not to modify a target protein to have a predetermined activity. Further, Blazquez *et al.* does not teach or suggest a method in which single amino acid residues are each replaced along the full-length of the encoded protein nor along the full-length of a pre-selected domain of the encoded protein. Furthermore, Blazquez *et al.* does not teach or suggest a method that includes a step of producing sets of nucleic acid molecules where, in each set, the nucleic acid molecules are produced by changing one codon in the target protein to a pre-selected codon, whereby the nucleic acid molecules in each set encode proteins that differ from the encoded proteins in another set by one amino acid. Blazquez *et al.* does not teach or suggest a method that includes organizing host cells into an addressable array in which the identity of each encoded modified protein is known. Giver *et al.* does not teach or suggest the elements missing from the teachings of Blazquez *et al.*

Giver *et al.*

Giver et al. does not teach or suggest the missing elements. Giver *et al.* teaches a method for evolving a protein to have increased thermal stability without loss of its activity at lower temperature. The method involves the steps of producing nucleic acid molecules encoding variants of an esterase **by gene random mutagenesis to produce a mixture of the** variants called a random mutant library, screening the expressed gene libraries in *E. coli* to produce colonies, which colonies are picked into 96-well microtiter plates. The process of random mutagenesis followed by screening was repeated six times. Giver *et al.* also states that **one or two mutations are introduced per nucleic acid molecule**, and that mutated molecules are expressed as a mixture.

Hence, the method of Giver *et al.* is completely different from the instantly claimed method. It differs in numerous steps. In the instantly claimed methods mutants are rationally produced by mutating one codon at a time to produce sets of nucleic acid molecules that each encode the same protein that differs from the target protein by one amino acid; separately expressing each set and separately screening the expressed proteins. In the method of Giver *et al.*, the mutated nucleic acids are produced by random mutagenesis, including gene shuffling, all variants are made in the same mixture and are expressed in the mixture, colonies are produced, picked and screened. The colonies may be placed in an array, but they are not are arrayed such that the identity of the encoded protein at each locus is known; each colony is indistinguishable from the next; whereas in the instantly claimed methods the identity of the nucleic acid molecule in the host cell and the encoded protein at each locus in the array is known.

Givers *et al.* states, page 12809, col. 2, that random mutations were introduced using mixtures of primers: "Random mutations were introduced during mutagenic PCR (14)." **The primers introduce *one to two* amino acid modifications per encoded protein,** and the modified nucleic acids are produced as a mixture resulting in sets of nucleic acids that encode mixtures (libraries) of proteins, in which members of the mixtures differ from the target protein by 1-2 amino acids. The sets do not contain nucleic acid molecules that encode the same protein nor do the proteins encoded by each set differ by one amino acid from the proteins in another set.

The Examiner states that the nucleic acid molecules were introduced into cells which were arrayed. It respectfully is submitted that this is not correct. The nucleic acid molecules, which contain mixtures of different proteins were introduced into cells and the cells were grown and colonies were picked and the plates were screened. Hence the proteins were not separately expressed and screened as required by the instantly claimed methods.

Analysis

Blazquez *et al.* does not disclose a method of directed no evolution, nor a method that includes any or all of the steps (a)-(d), common to all pending claims, as follows. For example, Blazquez *et al.* fails to teach or suggest a step of:

(a) producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, where the members of each set encode the ***same*** modified protein, and each encoded modified protein in a set differs from the encoded proteins in each other set

and from the target protein by one amino acid and where each codon is replaced along the full length of the encoding nucleic acid molecule or along a portion that encodes a domain of the protein.

Blazquez *et al.* teaches production modification of **seven** amino acid loci in TEM β-lactamases to assess the contribution each locus has on antibiotic resistance. Blazquez *et al.* is not performing directed evolution nor are sets of nucleic acid molecules produced in which each codon is replaced along the full-length of the protein or a domain thereof. Only 7 non-contiguous loci are replaced with the codon encoding the amino acid in the resistant phenotype. The loci are those that are modified in a resistant polypeptides and the change is the modification in the resistant polypeptides.

Blazquez *et al.* also fails to teach or suggest a step of:

(b) individually introducing each set of nucleic acid molecules into host cells to produce an addressable array of host cells, whereby the identity of each set of nucleic acid molecules in host cells of each locus in the array is known, wherein the cells of each locus of the addressable array contain the same modified nucleic acid molecules;

Blazquez *et al.* does separately express the seven polypeptides, but there only are made seven polypeptides. They are not introduced into a array, addressable or otherwise.

Blazquez *et al.* also fails to teach or suggest a step of:

(d) individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target, wherein:

each identified protein is designated a hit;

each hit contains a mutation designated a hit position; and

the predetermined property or activity is selected from among a chemical, a physical and a biological property or activity of the target protein.

Blazquez *et al.* **does not** screen the expressed proteins to identify proteins with altered activity (*i.e.* the loci that alter activity were known, and the altered activity was known, not evolved). Blazquez *et al.* tests the seven different proteins to study the effect of each change on the changed phenotype. Blazquez *et al.* does not even teach a directed evolution or screening method. Blazquez *et al.* teaches a study of seven polypeptides with modifications at loci that occur in naturally occurring loci. There is no evolution, modification of contiguous amino acids, nor is there screening.

Giver *et al.* does not cure these deficiencies. As discussed above, Giver *et al.* employs mixtures of oligonucleotides that in which **one to two** amino acids are modified in each encoded protein to produce mixtures of oligonucleotides. In the method of Giver *et al.* the mixtures of nucleic acid molecules are introduced into host cells and mixtures of proteins are expressed in the host cells. Thus Giver *et al.* **fails** to teach step (a) producing a population of sets of nucleic acid molecules that encode modified forms of a target protein, where the members of each set encode the same modified protein, and each encoded modified protein in a set differs from the encoded proteins in each other set and from the target protein by one amino acid and where each codon is replaced along the full length of the encoding nucleic acid molecule or along a portion that encodes a domain of the protein.

Giver *et al.* fails to teach or suggest step **(b).** In the instantly claimed method, the same protein is expressed in each set of host cells such that the identity of each protein is known *a priori*. As claimed the cells are arrayed such that the identity of the protein expressed in cells at each loci in an array is known. In the instantly claimed method, each protein is produced and expressed separately and is then introduced into host cells, the identity of the encoded protein in the host cells is known *a priori*. This means that in the instantly claimed method (1) it is not necessary to pick colonies; and to then (2) following screening, sequence the selected proteins to identify them or the hit positions.

In the method of Giver *et al.* the mixtures of nucleic acid molecules are introduced into host cells and are expressed in the host cells. In the instantly claimed method, the same protein is expressed in each set of host cells. Further, in the method of Giver *et al.*, the cells may be arrayed, but they are **not** arrayed such that the identity of the encoded proteins at each locus is known. In the instantly claimed method, each protein is produced and expressed separately and is then introduced into host cells so that he identity of the encoded protein in the host cells is known. This means that (1) it is not necessary to pick colonies; and (2) following screening, the selected proteins do not have to be sequenced to identify them or the hit positions.

(c) Individually screening each set of encoded proteins, whereby one or more proteins that have a predetermined property that differs from the target protein is/are identified, where all of the encoded proteins in each set have the same modification; and the proteins in each set differ from the proteins in another set by one amino acid and from the target protein by one amino acid.

As discussed, in the method of Giver *et al.* the modified proteins differ from the target protein by one or two amino acids. The instantly claimed methods require that the modified proteins differ by only a single amino acid.

(d) Individually screening each set of encoded proteins, whereby one or more proteins that have a predetermined property that differs from the target protein is/are identified so that is hits are identified. By performing the method of Giver *et al.*, the colonies of cells are picked and screened, but the since they are not addressably arrayed, positives must be sequenced to identify them.

Hence, the method of Giver *et al.* is completely different from the instantly claimed methods of independent claims 1, 22-24, 27 and 30, as well as any dependent claims. Giver *et al.* fails to disclose elements of the claimed methods, including, but are limited to, one or more of the steps (a)-(d): producing sets of nucleic acids in which each set encodes the same protein that differ from each other set by one amino acid, individually introducing each set of nucleic acids molecules into different host cells, addressably arraying host cells that include nucleic acids that express the same protein; separately expressing and screening each encoded protein.

Analysis

The combination of teachings of Blazquez *et al.* and Giver *et al.* fails to result in the method of any pending claim. Since Blazquez *et al.* fails to teach at least steps (a), (b) and (d), and Giver *et al.* fails to teach any of steps (b)-(d), the combination of teachings of Blazquez *et al.* cannot and does not result in the method of claim 1. Each of the dependent claims the other independent claims, and their dependent claims, references similar steps. T. Therefore the combination of the teachings of Blazquez et al. and Giver et al. does not result in any of the instantly claimed methods. There is nothing taught or suggested in Giver *et al.* that would have lead one of ordinary skill in the art to have modified the method of Giver *et al.* to produce any of the instantly claimed methods. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

Claims 7, 24-31 are rejected under 35 U.S.C. §103(a) as being unpatentable over Blazquez *et al.* in view of Giver *et al.* and further in view of additional references that are alleged to teach elements of the rejected claims. The additional references do not supply the teachings missing in the combination of Blazquez *et al.* in view of Giver *et al.* Since the combination Blazquez *et al.* in view of Giver *et al.* does not result in the claimed methods of

the independent or base claims, these rejections will be addressed by addressing the rejection of the independent and base claims.

The rejection of claims 7, 24 and 27-29

Claims 7, 24, and 27-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Blazquez *et al.* in view of Giver *et al.* and further in view of Berlioz *et al.* (U.S. Patent No. 5,925,565) because, while Giver *et al.* allegedly only fails to teach using eukaryotic cells or assessing the titer of the viral vectors, Berlioz *et al.* teaches "assessing the titer of the viral vectors after transfection for each set of eukaryotic cells (column 14, lines 39-65) and where the viral vector encodes for a protein involved in viral replication (column 5, lines 35-65)." The Examiner concludes that it:

> would have been obvious at the time of the invention to combine the method taught by modify the methods taught by Blazquez *et al.* and Giver *et al.* with Berlioz *et al.* in order to study the effects of the protein in an eukaryotic setting. Berlioz *et al.* teaches a method that allows eukaryotic cells, such as a human cell, to express a desired protein (column 6, lines 5-22)for the purpose of producing a therapeutic treatment (column 7, lines 15-25). Giver et al.'s and Blazquez et al.'s methods teach screening for different proteins that exhibit a desired biological, chemical, or physical property. Thus one of ordinary skill in the art seeking to create a new therapeutic: treatment, would be motivated to use Giver et al. and Blazquez et al.'s methods to design a product and use Berlioz et al.'s method to express the protein in an eukaryotic cell.

This rejection respectfully is traversed.

Claims

Claim 7 is directed to the process of claim 1, where the nucleic acid molecules comprise viral vectors; and the cells are eukaryotic cells that are transduced with the vectors.

Independent claim 24 includes the method of claim 1 and recites that the nucleic acid molecules are in viral vectors and the titer of each set of vectors is assessed at step (b).

Claim 27 similarly recites that the nucleic acid molecules are in viral vectors and the titer of each set of vectors is assessed at step (b) and recites additional steps.

Analysis

As discussed above, the combination of teachings of Blazquez *et al.* and Giver *et al.* fails to disclose, teach or suggest teach any or all limitations of any of claims 1, 22-24, 27 and 30, and hence fails to disclose, teach or suggest any or all elements of the rejected dependent claims. Berlioz *et al.* fails to cure the deficiencies of Giver *et al.*, since Berlioz *et al.* does not teach or suggest a method that includes producing a population of sets of nucleic

acid molecules that encode modified forms of a target protein, where all nucleic acid molecules in a set encode the same modified protein and the proteins are modified along their full-length or the full-length of a domain (step (a)); arraying cells containing the nucleic acid molecules such that the identity of the encoded protein is known *a priori*(step (b)), and/or individually screening each set of encoded proteins to identify one or more proteins that have a predetermined property that differs from the target protein is/are identified (step (d)). Therefore the combination of teachings of Blazquez *et al.* Giver *et al.* and Berlioz *et al.* does not result in the methods of claims 7, 24 and 27-29.

The rejection of claims 25 and 26

Claims 25 and 26 are rejected under 35 U.S.C. §103(a) as being unpatentable over Blazquez *et al.* and Giver *et al.* further in view of Rivet *et al.* ((2000) Gene Therapy 7:924-929) because, Rivet *et al.* allegedly teaches real time virus titering (page 925) and using tagged replication and expression enhancement (page 926, right column), and hence cures the deficiencies in the teachings of Giver *et al.* and Berlioz *et al.*, which fail to teach real-time virus titering or tagged replication and expression enhancement.

The Examiner concludes that:

> [i]t would have been obvious to one of ordinary skill in the art to combine the methods of Blazquez *et al.*, Giver *et al.*., Berlioz *et al.* and Rivet *et al.* in order to gain the benefit of determining the effectives of the viral vectors. This rejection respectfully is traversed.

Claims 25 and 26 are directed to the methods of claim 24, in which titering is effected by real time virus titering, As discussed above, the combination of teachings of Blazquez *et al.* and Giver *et al.* fails to teach numerous elements of the independent claims, which Berlioz *et al.* fails to cure the deficiencies in the teachings of Giver *et al.* . Rivet *et al*, not only **does not teach** real time titering as claimed in claim 25 or claim 26, also does not cure the deficiencies in the teachings of Giver *et al.* and Berlioz *et al.* Hence, the combination of teachings of Blazquez *et al.*, Giver *et al.*, Berlioz *et al.* and Rivet *et al.* does not result in the methods of claims 25 and 26. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

The rejection of claims 30 and 31

Claims 30 and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Blazquez *et al.*, Giver *et al.* ((1988) *Proc. Natl. Acad. Sci. U.S.A. 95:* 12809-12813) in view of Persson *et al.* ((1985) *Journal of Virology 54*:92-97) because Persson *et al.* is alleged to

teach a method that uses a Hill analysis for determining the rate in which host cells are infected with viruses (abstract, page 94, left column). The Examiner concludes that:

> [i]t would have been obvious to one of ordinary skill in the art to combine the methods of Giver *et al.* and Persson *et al.* to gain the benefit of deterring if the plasmids or vectors are infecting the host cells.

This rejection respectfully is traversed.

Claim 31, which is dependent on claim 30, which includes the elements a)-d) as discussed above, and further includes recites that the "performance of the screened proteins is evaluated by a Hill analysis. Persson *et al.* does not teach or suggest a Hill analysis as claimed, nor does Persson *et al.* teach or suggest the elements of claim 30, missing from the combination of teachings of Blazquez *et al.* and Giver *et al.* Therefore, the combination of teachings of Giver *et al.* and Persson *et al.* does not result in the methods of claims 30 and 31. Therefore for these reasons and those discussed above, the Examiner has failed to set forth a *prima facie* case of obviousness of any of the pending claims.

* * *

In view of the above, reconsideration and allowance respectfully are requested

Respectfully submitted,

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